Ernst. (H. C.)

A CONSIDERATION

OF THE

BACTERIA OF SURGICAL DISEASES.

Read, by invitation, before the American Surgical Association

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Presented by the author

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VERY many observations have been made, extending over a long space of time and by many different observers, with the object of determining the etiology of the pus-forming processes in man. Before our knowledge of bacteriological methods was as precise as is now the case, such experiments as those of Councilman (*Virchow's Archiv*) and others were considered to be conclusive. These experiments showed, or seemed to show, that the introduction of simple irritant substances into the animal tissues was sufficient to set up a more or less marked degree of irritation, followed by suppuration and the production of a localized or sometimes of a general purulent process. The materials used were croton oil, turpentine, and the like, and the results observed after their introduction into animals were ascribed to a specific power which they possessed, which could, and generally did, produce pus-formation when such materials came in

contact with animal tissues. These experiments seemed conclusive at the time they were published, but, with our more recent and intimate knowledge of the bacteria, their fallacies are easily to be perceived, and are seen to lie very largely in the imperfect methods taken to prevent the activity of bacteria, and the fact that these latter were not sought in the lesions produced by means which would lead to their detection if present. It is not surprising, therefore, to read the results of Klemperer in his admirable paper upon this subject (*Zeitschr. f. klin. Med.*, Bd. x. S. 198, and *Fort. d. Med.*, 1886, S. 34). Thinking, and with apparently good reason, that the results of other observers might be due, in part at least, to the imperfect sterilization of the *skin* of the animals experimented upon, this author made all his inoculations at a point which had been just previously seared with a red-hot iron, and again used the actual cautery at the point of inoculation after the operation was over. Of course, the syringe and material employed were thoroughly sterilized in the usual way. Experiments were made in large numbers, with acids, alkalies, cantharides, mustard oil, petroleum, and other irritating materials, and upon rabbits, guinea-pigs, and mice. Pus-formation *never* occurred: at the most, the only sign of any local or general disturbance being a moderate localized serous effusion. After the use of croton oil or mercurial injections, there occurred in some cases a firm ne-

crotic (diphtheroid) infiltration,—Weigert's acute coagulation-necrosis. In only three of a large number of cases did suppuration occur, and in all of these cases micrococci were found in the pus, and the observations were therefore considered imperfect.

The observer concludes that the strongest chemical cannot produce suppuration without the presence of bacteria, and his results certainly seem to support this view. He found also a micrococcus, which under ordinary circumstances would produce no suppuration upon inoculation into animals, but which would set up a flow of pus if an irritation were produced such as would result after the introduction of acrid materials into the tissues.

Scheuerlin (*Von Langenbeck's Archiv*, Bd. xxxii. Heft 2) has also investigated this question, and has come to the same conclusion as Klemperer, that no suppuration will occur without the presence of micro-organisms. His method of experimentation was to place from one to four drops of the irritating substance in capillary tubes. These tubes were sealed, sterilized in the moist chamber, and introduced under the skin of rabbits with all possible antiseptic precautions. When the incision made for their introduction was thoroughly healed, taking six days as a rule, the tubes were broken under the skin, and the animals were killed in from four to eight days. Thirteen irritating substances were used, such as oil of turpentine, croton oil,

oil of mustard, formic acid, etc., with distilled water as a control. In only one case was suppuration observed, and bacteria were found in this one.

The point being determined, therefore, that there is at least a very strong probability that no suppuration occurs without the presence and activity of bacteria, the study of the organisms concerned in these processes becomes at once of great interest.

Pasteur was one of the first to investigate the subject, and his *vibrion septique* was one of the first of the organisms connected with a septic process with anything like certainty (*Bull. de l'Acad. de Méd.*, 1877, p. 793). Since that time "the micrococcus of pus" and like expressions have been commonly employed as denoting a single organism which might be held responsible for all purulent processes, but with unreasonable inaccuracy, as later investigations have shown. In other words, there is not one but a number of pus-producing bacteria, which have been fairly well identified and described, and there is no reason to suppose that this number will not be added to as the investigations in this direction are proceeded with.

Rosenbach was the first observer to differentiate more than one variety of pus-forming micro-organism in man ("Micro-organismen bei den Wundinfektionskrankheiten des Menschen," 1884), and was quickly followed by Krause, Passet, Hoffa (*Fort. d. Med.*, 1886, S. 75), and others,

working in the same direction. Of course, the pioneer in this as in most other of the exact work in modern bacteriology was Koch, who gave indications of the direction and methods of research in his classic "Wundinfektionskrankheiten."

Of the bacteria concerned in pus-formation which have been found in the human body, and shown to possess pathogenic properties by inoculation experiments upon animals, there are at least twelve varieties which have been described with sufficient accuracy to make it possible for any observer to identify them. This identification, however, is not a simple matter of staining reaction and microscopic observation, as is the case with the bacillus of tuberculosis, but includes all of the means adopted for the classification of micro-organisms from a bacteriological point of view,—not, however, from a botanical stand-point.

The "points" of any bacterium, which form collectively the diagnostic evidence of its place in the classification, are: 1, its form, size, and arrangement; 2, its power of motion or not; 3, its behavior upon the three great classes of culture-media, nutrient gelatin, nutrient agar-agar, and solidified blood-serum, including the form and color of the colonies, and its appearance upon plate-cultures; 4, the temperature at which it flourishes best; 5, the rapidity of the development of the colonies; 6, whether it produces spores or not; 7, whether or not air is necessary

for its growth,—aërobic or anaërobic; 8, whether it produces gas or not; 9, its behavior in gelatin,—that is, whether it does or does not liquefy the nutrient gelatin; 10, its behavior towards staining-materials; 11, its pathogenic properties when inoculated into the lower animals.

All of these points must be investigated when it is desired to obtain complete evidence in regard to any special bacterium, and sometimes it is necessary to go through them all before it can be determined that it is one rather than another variety which may be under observation. From this statement it may be easily seen that it is occasionally a difficult matter to say with what organism a given observer has been working.

Rather than to run over all the work of each one separately, in order to give a clear idea of what has been done by the various observers in this line of investigation, I have thought it best to give a summary of the various bacteria thus far found in the "infectious wound-diseases" of man, according to the above method of classification. In this it seems to me as if a clearer idea of them could be conveyed than by any other hasty method.

1. In the first place comes the Staphylococcus pyogenes aureus, very abundant in pus, first described by Rosenbach ("Mikroorganismen bei den Wundinfektionskrankheiten des Menschen") and Passet ("Aetiologie der eiterigen Phlegmonen des Menschen"). They are cocci of variable size,

occurring in masses, and often as diplococci, of an average diameter of 0.87 μ. On agar-agar at 37° C. they grow as a glistening raised colony, two to four millimetres broad, of a bright orange color. This color may often appear only after some time, and is then accompanied by a smell like that of sour paste. In gelatin there is generally a cloudy, grayish streak, which after about three days becomes of a yellowish, finally orange color, and liquefies the gelatin, into which the colony sinks. By a needle plunged into agar-agar there is presented a cloudy, beaded yellowish line, which becomes opaque after twenty-four hours, and later of an orange color. There is a layer three to four millimetres broad, extending over the surface of the agar-agar about the point of entrance of the needle. On sterilized potato the colony has an orange-yellow centre, with a thin, whitish, almost transparent border, which gradually becomes thicker and yellow. There is also the strong paste-smell. Its growth in blood-serum is exactly as upon agar-agar. It grows best at from 30° to 37° C., somewhat more slowly at summer temperature. It retains its vitality for a long time without oxygen, and produces no gas-formation. It liquefies nutrient gelatin and produces an orange-yellow pigment. Injected into the peritoneal cavity of rabbits and other animals, it will produce death after twenty-four hours, or else a tremendous suppuration which may last ten days or more.

This and the micrococcus of osteomyelitis (Becker, *Deutsch. Med. Woch.*, 1883, No. 46) are probably identical, because the behavior of the two under cultivation is the same; they are the same under the microscope, and the results after inoculation are the same.

2. The Staphylococcus pyogenes citreus found in pus and described by Passet (*l. c.*) has precisely the same properties as the preceding, except that its colonies are colored lemon-yellow instead of orange-yellow. It is not nearly so common as the Staphylococcus aureus.

3. The Staphylococcus pyogenes albus (Rosenbach, *l. c.*) occurs in pus less frequently than the Staphylococcus pyogenes aureus, and has the same morphological characteristics and similar pathogenic properties, but in a milder form, producing as a rule only localized abscesses when introduced under the skin of the lower animals.

4. The Micrococcus pyogenes tenuis (Rosenbach, *l. c.*) occurs in large subacute abscesses mostly, and is a form of micrococcus somewhat larger than the staphylococci, without regular arrangement. It grows on the surface of agar-agar, as a thin, almost transparent layer, and in the substance of the same material its growth is almost imperceptible. It grows very slowly, does not liquefy gelatin, and occasionally some of the larger ones are stained at the two poles by the aniline dyes employed, leaving the centre almost clear.

5. The Staphylococcus cereus albus (Passet, *l. c.*), found in pus, is a micrococcus of irregular size, occurring in masses or occasionally in chains; it grows on plate-cultures with nutrient gelatin as white points one to two millimetres broad. In gelatin the surface of the colony is grayish white, with thick stearin-like drops along the needle-track. On potatoes there is a grayish-white colony of medium thickness, and on blood-serum a grayish-white dull streak. It does not liquefy gelatin.

6. The Staphylococcus cereus flavus (Passet, *l. c.*) is exactly like the preceding, except that it produces a lemon-yellow color somewhat darker than that of the Staphylococcus pyogenes citreus previously described. It is of very rare occurrence.

7. Bacillus saprogenes No. I. (Rosenbach, *l. c.*) is a rather large bacillus containing prominent spores, found by Rosenbach in the white plugs of the follicles of the tonsils. On agar-agar the colony appears as a yellowish-gray, opaque streak, about one millimetre thick, of cheesy, sticky consistency, with irregular edges after some time, and after four or five weeks a foul smell like that of sour cake. It is of very slow growth, produces large spores, and gives out an intensely foul odor when free access of air is allowed; when air is excluded the growth is retarded and the foul smell is delayed in making its appearance. Inoculation into

the knee-joint of a rabbit gave a negative result.

8. The Bacillus saprogenes No. II. (Rosenbach, *l. c.*), found in the material from foul-smelling feet, is somewhat smaller than the preceding, and after twenty to twenty-four hours the colony on agar-agar appears as numerous fine points, which enlarge and coalesce, and form first an irregular clear, and then opaque, tough, slimy layer. The smell is that of foul feet. It grows very rapidly, is aërobic and also facultatively anaërobic, and gives its characteristic odor much more quickly by the free access of air than when this condition is wanting. It seems to have mild invasive and pyogenic properties.

9. Bacillus saprogenes No. III. (Rosenbach, *l. c.*), found in the pus of septic gangrene, on agar-agar after eight days at summer temperature, appears as a colony three millimetres broad, of an ash-gray color, not sticky, but of an almost fluid consistency, with a rounded contour. It grows with medium rapidity, reaching its maximum in eight days. There is very rapid fouling of albumen with free access of air; without this the process is slower. All culture-media except milk produce this foul smell in the presence of this organism. After injection, either subcutaneously or into the joints of a rabbit, there occurred a yellowish-green infiltration with a surrounding redness, as well as the characteristic foul smell.

10. Bacillus pyogenes fœtidus (Passet,

l. c.) was found in abscesses about the anus, and consists of short staffs with rounded ends, often occurring two or more together, with an average length of 1.45 μ, and a breadth of 0.58 μ. It has slight motion. Plate-cultures in gelatin show after twenty-four hours white points, later becoming grayish-white. Magnified twenty diameters, the colonies appear thick and white in their centres and thick and gray at the edges. In gelatin in tubes, after twenty-four hours there is a soft, grayish-white, slimy layer on the surface, with rather thick, irregular edges. The needle-track is softened at first, and later presents large and small points all along it. Old cultures cloud the gelatin at the upper portions. On potato there is a glistening luxurious growth of a clear brown color; on blood-serum, a thick grayish-white streak. It grows rapidly, and does not liquefy nutrient gelatin. Subcutaneous injections and those into the abdominal cavity produce death in guinea-pigs and mice after twenty-four hours. The bacilli are found in the blood, but not at the point of inoculation nor in the organs.

11. Streptococcus pyogenes (Rosenbach, *l. c.*) was found in the pus of an erysipelas-like process. It is a micrococcus occurring in chains of from two to thirty, and of from 0.58 to 0.73 μ in diameter. On plate cultures in gelatin it produces fine round granular points. In a streak of the needle the culture is thickest at the middle, of a light brown color; its contour be-

comes thicker, swells up, and finally assumes a terraced form. Needle-punctures in gelatin show a delicate layer around the entrance of the needle, the needle-track itself presenting fine points of growth, at first very sparse, but later larger and much more numerous. In agar-agar at 35° to 37° C. there is a string-like, grayish-white line with points all along it, and no surface-layer. On cooked potato it does not grow well. On blood-serum it grows along the needle-track as a thin, string-like colony. It grows best at 35° to 37° C., much more slowly at summer temperature. It grows slowly, the colony being but two to three millimetres broad after two or three weeks in surface-culture. After four months the culture is almost dead. It may be renewed in fresh nutrient material. It will decompose albumen in a vacuum, does not liquefy gelatin, and upon inoculation in animals produces a very severe spreading, erysipelas-like suppuration.

12. Streptococcus erysipelatis (Fehleisen, "Die Aetiologie des Erysipelas"), found in the lymph-channels during erysipelas, is a small micrococcus occurring in pairs or in chains, especially upon cultivation in bouillon, of about 0.3 to 0.4 μ in diameter. On plate-cultures with gelatin it occurs in fine round granular colonies. Needle-cultures in gelatin after twenty-four hours show very fine white points or flecks the length of the needle-track, which later become a homogeneous white streak. There is either no, or very slight, growth

upon the surface of the gelatin around the point of entrance of the needle. In agar-agar it grows very sparsely over the whole surface, in small, almost imperceptible colonies. On blood-serum at 37° C. the colony is almost exactly of the color and nearly indistinguishable from the serum itself. It develops best at from 20° to 30° C., grows slowly, and produces in rabbits a sharply-defined, spreading redness, without suppuration. It always occurs in the lymph-channels of the skin in erysipelas. It has been tried in a number of cases on man, and in these produced a typical erysipelas.

13. Bacillus pyocyaneus (Gerrard, "De la Pyocyanie et de son Microbe," 1882), found in green pus, is a short, fine rod, very likely to be mistaken for a micrococcus. In plate-cultures with nutrient gelatin, after two or three days the whole of the plate has a clear green tint, the colonies liquefying the gelatin in a funnel-shaped depression. Under a low power, the colonies appear round, somewhat yellowish, and glistening with clear granular edges.

Needle-cultures in gelatin after twenty-four hours liquefy the upper layer of the gelatin in a funnel-shaped depression, with a fluorescent coloring of the upper portion. The liquefaction later extends out to the side of the test-tube, and the whole of the gelatin takes on this clear fluorescent appearance. In agar-agar there is a moist greenish-white colony, whilst the

whole of the agar-agar is colored with a diffuse fluorescence. On potato dry colonies of a rust-brown color appear, which turn green with ammonia and red with acids. It grows rapidly; will not grow under mica (glimmerplatte), and liquefies nutrient gelatin. Guinea-pigs die after cultures of this organism have been injected into the abdominal cavity (Koch). Rabbits survive its injection into the blood-current.

14. Pneumoniacoccus-like bacillus (Pneumokokken ähnlicher Bacillus, Passet), an organism found in pus, occurring as micrococci,—very rarely as short staffs,—in contradistinction to the pneumoniacoccus of Friedländer, which very often has this form. On plate-cultures small grayish-white nodules appear, made up of the cocci. Needle-cultures in gelatin show after twenty-four hours a grayish-white hemispherical nodule on the surface, with no growth in the body of the nutrient soil. After three or four weeks there appears a brownish color in the gelatin, and a foul odor. On potato, at 37° C., there is a thick, soft, whitish, glistening layer, with no gas-production. Its growth is rapid, it produces no gas, and does not liquefy gelatin. Injected into serous cavities it produces a suppurative process, but has almost no effect after subcutaneous inoculation. It has no result at all in inhalation-experiments.

This summary includes all the organisms

which have yet been described with a sufficient degree of accuracy to permit of their classification, and which have been found in the purulent or inflammatory processes in man. It is made up from the work of the various observers spoken of in the earlier part of the paper, and is based upon the excellent tables of Dr. Eisenberg. It does not, of course, include those organisms described by Koch in his "Wundinfektionskrankheiten," because they were connected solely with the lower animals by that observer. The summary gives us, however, a very comprehensive view of the work that has already been done, and of the number of organisms which have already been found in the various suppurative and inflammatory processes occurring in man. Unquestionably the subject is not yet exhausted, and additions to the list may very probably be made.

I have been interested in examining such cases as I could gain access to of graver forms of the suppurative process than simple acute abscesses and the like. These latter have been investigated in such numbers by the observers already alluded to—Rosenbach, Krause, Passet—that it hardly seemed worth while to go over exactly the same ground again in the same way. I have selected therefore, so far as was possible, severe cases of long standing and those where there had been no access to the air previous to the time of examination. Cases of this sort, fulfilling all

the indications required, are not especially easy to find, and my thanks are due to Drs. J. C. Warren and E. H. Bradford for the material which they have placed at my disposal.

In chronological order the cases which I have examined are as follows:

I. Cultivations were made from an abscess of slow growth occurring at the seat of an ununited fracture of the left humerus of over a year's standing. No external injury to the parts was apparent, and there was no opening until the incision was made through which the pus was obtained for cultivation. Cultures were made from the pus which flowed out and from the wall of the abscess. Colonies of the Staphylococcus aureus and albus made their appearance upon the nutrient material in the course of a few days, were separated from each other, and carried through a number of generations. This case is interesting because the diagnosis of tuberculosis was made from the clinical symptoms, but a very careful examination of cover-glass preparations of the pus and of scrapings from the wall failed to show any indication of tuberculosis either in the way of giant-cells or of the bacilli of tuberculosis.

II. A large perinephritic abscess of several weeks' duration. Cultivations made from the pus and from the abscess-wall showed the presence of the Staphylococcus aureus and albus, which were separated and grown for some time. The case ter-

minated fatally from pyæmia, and micrococci corresponding to those in the cultivations were found in microscopic preparations of the valves of the heart, in the kidney, spleen, and blood.

III. A skin-disease of the knee in a girl of 20, of some years' standing. An incision was made through the growth, a flap turned back, and cultivations made from beneath this by scraping the needle over the surface. The colonies which made their appearance were the Staphylococcus citreus and albus, which also were separated and carried through a number of generations. Sections were made of a portion of the diseased skin of the knee which had been put into absolute alcohol at once after removal. Micrococci corresponding in size to those in the cultures were found in large numbers just below the layer of flat epithelium, with colonies running down at frequent intervals between the cells into the deeper layers of the skin. The microscopic appearances in gross showed the presence of giant-cells and granulation-tissue, with some proliferation of the epithelial cells into the deeper parts with a few clusters of epithelial cells. The most careful search through a number of sections, by Dr. W. F. Whitney and myself, failed to show the bacilli of tuberculosis.

IV. Perinephritic abscess of several weeks' duration, with a small opening which was only a few hours old. The abscess was opened by an incision through

the skin made upon the opposite side of the body from this sinus, and cultivations were made upon agar-agar from the pus thus obtained. Pure cultures of the Staphylococcus aureus made their appearance in the course of a few days.

V. Erysipelas of the foot four days old surrounding a penetrating wound. An incision through the skin at a point just beyond the line of redness was made, and cultivations were sown with a platinum-needle from over the bottom of the wound. A pure culture of the Erysipelas coccus was obtained.

VI. Cultivations were obtained from the material in the vagina of a case of vaginal diphtheria after parturition at the Boston Lying-in Hospital, through the kindness of Dr. W. L. Richardson. There appeared, in the course of a few days, colonies of the Staphylococcus albus, and of the Bacillus pyogenes fœtidus,—the latter of which I had hoped to be the first to describe, but which will be seen, by the preceding mention of it, to be credited to Passet.

VII. A case of vaginal diphtheria occurring under the same circumstances as the preceding. Cultivations made in the same manner were productive of colonies of the Staphylococcus albus only. The case was almost convalescent at the time the examination was made.

VIII. A third case of vaginal diphtheria under like conditions as the two preceding, with the exception that there was

a considerable amount of pus present in the vagina. This patient was also convalescent. The culture-tubes showed the presence of the Staphylococcus aureus and albus, and of the Bacillus pyogenes fœtidus. There was also present a bacillus resembling the Bacillus subtilis, which was probably due to some contamination occurring during the manipulations.

IX. A case of psoas abscess in a child; a large amount of pus. Opened under carbolic spray and cultivations made from the pus and from the abscess-wall. The result showed a very free growth of the Staphylococcus albus only.

X. A case of hip-disease in a child, with a profuse suppuration about the hip-joint. Cultivations made from the pus as it flowed from the incision and from the joint itself showed the presence of the Staphylococcus aureus and albus. The latter—Staphylococcus albus—was much the most prominent, Staphylococcus aureus appearing in only one tube out of ten in which cultivations were made.

XI. A case of hip-disease in a child, with sinus which had been open for some time. In this case cultivations were made from a fresh opening made to secure drainage, and a free development of the Staphylococcus aureus and albus, and of a fine oval bacillus with no pathogenic properties, was obtained; the latter was probably the so-called Bacterium termo. The Streptococcus pyogenes was also found in this case, making four in all which were obtained.

XII. A case of glanders in the human subject. The body was covered with a large number of pustules, three or four of which were opened and cultivations upon agar-agar and in gelatin were made, the point at issue being to discover whether any of the ordinary forms of bacteria present in pus would be found in this case. As a result, colonies of the Staphylococcus aureus and albus were obtained, as well as two or three colonies of a fine bacillus answering the description of the organism found in this affection by Gaffky.

XIII. A case of lumbar abscess, with possible caries. Cultivations made from the pus flowing out after incision of the abscess resulted in the development in a few days of a number of elevated, glistening, yellowish colonies, which, upon microscopic examination, were found to be made up of pure cultures of very fine, short, straight bacilli.

XIV. Psoas abscess in a child. Cultivations from the pus obtained in this case showed the presence of the Staphylococcus cereus flavus of Passet, so called, and of the Staphylococcus aureus.

XV. Psoas abscess in a child. Cultivations from the pus of this case gave, as a result, colonies of the Staphylococcus albus, and of a bacterium of which a more detailed description will be given farther on.

XVI. A case of abscess of the arm in a man. There was extensive purulent infiltration over the region of the shoulder

and upper arm, extending into the left side, and penetrating into and between the various muscles of the affected parts. A number of cultivations were made with the pus as it flowed out from the freshly-opened wounds, and in the course of a few days very beautiful colonies of the Streptococcus pyogenes were obtained.

XVII. Abscess of the back following caries of the spine, which had existed for some time. The abscess was apparently the size of the fist, of rapid growth, and was situated in the right lumbar region very near the spine. Cultures were made from this case as before, from the pus flowing from a freshly-made incision. After a time, slowly-developing colonies of the Micrococcus pyogenes tenuis were seen to make their appearance, together with colonies of the Staphylococcus pyogenes albus.

XVIII. A case of bubon d'emblée. The swelling was of slow growth, and absolutely no history of venereal infection could be obtained. There were signs of pulmonary solidification, however, and the question as to whether the swelling in the groin might be of tuberculous origin or not became of interest. Upon incision into the tumor, a thick, dark pus was forced out, and cultivations were made, which, in the course of a week, produced colonies of the Staphylococcus pyogenes albus and of the Micrococcus pyogenes tenuis. Two of the glands which were enlarged to the size of marbles were placed in absolute alcohol, and upon several sub-

sequent occasions sections were stained and examined with the greatest care for the presence of bacillus of tuberculosis; the results of such examinations were in all cases negative.

This completes the list of the cases which I have examined. It is not an extensive one, and was purposely, so far as possible, limited to the more chronic processes, and to those where there was and had been no access to the air until the moment of time during which the cultivations were completed. It is, however, important to observe that in every case examined one or more forms of bacterial life were developed upon the nutrient material, and therefore that, so far as these observations go, they tend to refute the assertion that bacteria are not present in the more chronic forms of purulent disease. It is true that they may not be discovered by simple microscopic examination of the fresh pus, but this does not prove that they are not present. Their numbers may be so small that a few cover-glass preparations made up from only a portion of a drop of the pus might easily fail to reveal their presence. Cultivations, however, offer a means for their development, and the presence of but one or two bacteria may be demonstrated in this way in cases where they might be overlooked if any other method of investigation were employed.

In all the cases that I have reported the mode of procedure was as follows,—ex-

cept, of course, in the diphtheritic vaginæ, where there was necessarily more or less free access to the air. After anæsthesia was complete, the skin over the point selected for the incision was thoroughly washed in a solution of corrosive sublimate, one to one thousand; the surgeon's hands were sterilized in the same solution. The incision was made with a knife freshly removed from the same solution, and as soon as the pus-containing cavity was opened, a platinum needle taken from the flame of an alcohol-lamp was plunged into it, and test-tubes containing nutrient gelatin and nutrient agar-agar were inoculated with the material obtained. The tubes were then removed to the laboratory, and in some cases plate-cultures were made at once, and in others not until twenty-four hours afterwards. A number of the tubes were also placed in the incubator at 37° C., and the conditions of development were observed under these circumstances. Every one of the bacteria obtained was identified by means of its microscopic appearances, and its behavior under cultivation in various nutrient media on plate-cultures with nutrient gelatin, on agar-agar, etc., so that no name is given to any of the organisms found in the cases reported without a sufficient basis of careful observation. A large number of the bacteria were propagated through from fifteen to twenty generations, and were found to preserve their propertiesunder cultivation during the whole of the time they were under

observation, extending from six months to over a year. Owing to my absence abroad during last fall and an unfortunate mistake in regard to the time of my return, a number of the cultures were lost through drying. By this I do not mean that all of any one variety were lost, but that about one-half of the collection I had made from different cases were destroyed. This was to be regretted, because I had kept every bacterium from every case separate, and had carried each one through a long number of cultivations, extending over a great length of time, with a view to the determination of differences, if any existed, in the pathogenic powers of micro-organisms from different cases, but of the same apparent nature, as shown by their appearances under varying conditions of cultivation. This point the accident which I speak of prevented me from investigating, although the results obtained by other authors seem to indicate that there is no such difference as had seemed to me possible when I began this research; and the fact seems to be established that the differences in the pathological changes produced by what we must call the same bacteria are the result of influences other than those peculiar to the bacteria themselves, such as changes of nutrition and method of infection. It is to such differences as these that the varying clinical results produced by the Staphylococcus pyogenes aureus, as seen in osteomyelitis and acute abscesses, must be

ascribed; for it seems to be fairly well established that the organisms producing the first and part of the latter processes are identical. The methods of Koch—including of course the employment of solid culture-media—have been used in all cases, as being conducive to greater precision and more definite results than any others that are known. This is especially the case in such an investigation as the present one, for without these methods the differentiation of the various bacteria in the pus-producing processes would be practically impossible.

Inoculation experiments have been made in considerable number to test the pathogenic powers of the various organisms which I have had under observation. Here, again, the method employed has been that of Koch. The place selected for the inoculation has been first thoroughly cleansed and then soaked in a solution of corrosive sublimate (one to one thousand), the hands of the operator have been sterilized with the same solution, and for inoculation a syringe like the one I have here has always been employed. It may be readily taken apart and cleansed, has no packing or other parts to become foul, and may be subjected to any temperature required for sterilization. In all cases these syringes have been subjected to 150° C. for an hour just before being used. The material for inoculation has been prepared in the case of cultures in gelatin media by simply melting the gelatin, pouring it into a ster-

ilized capsule, and then using the required quantity in the syringe shown ; or, when the cultures were upon nutrient agar-agar, by placing a portion of the culture in boiled distilled water and using this in the same way as the liquefied gelatin cultures.

The animals used were all selected for their apparent condition of good health, had all been under observation for some time before the operations, many of them having been kept in the country miles away from other animals, and were all kept under the very best possible hygienic conditions. The result of these precautions is seen in the fact that in no single case did the post-mortem examination reveal any abnormal condition of affairs that might not fairly be attributed to the activity of the bacteria which had been injected.

The animals employed were guinea-pigs. Mice were excluded because symptoms produced in them are not fairly comparable to those obtained in larger animals, and rabbits could not be obtained in Boston at any price during the time that I was carrying on my inoculation experiments.

The bacteria with which I have experimented are the following:

The Staphylococcus aureus and albus.
The Staphylococcus pyogenes citreus.
The Erysipelas coccus.
The Streptococcus cereus flavus.
The Streptococcus pyogenes.
The Micrococcus pyogenes tenuis.
The Bacillus pyogenes fluorescens.

The records of the inoculation experiments are as follows:

Inoculation experiments with the Staphylococcus pyogenes aureus.

I. A full-grown healthy guinea-pig inoculated in a vein of one leg made prominent by partial constriction. Two drops of a pure culture in gelatin of the sixteenth generation of the Staphylococcus aureus from the first case reported (ununited fracture of the arm) were employed. The cultivations had been conducted out of the body for over a year. Death occurred in less than twelve hours. The autopsy showed very little change in the internal organs: the heart was much distended and full of fluid blood. Cover-glass preparations showed the blood to contain numerous micrococci, and cultivations made from the blood in nutrient gelatin showed the presence of the Staphylococcus aureus.

II. An adult guinea-pig inoculated with the same culture as the preceding, under the skin of the abdomen, two cubic centimetres of the cultivation in gelatin being used. Killed in ten days. An abscess the size of a marble, filled with thick yellowish pus, was found just under the point of inoculation. Cover-glass preparations of this pus and sections of the abscess-wall showed the presence of large numbers of micrococci. Cultivations were made in gelatin and upon agar-agar (37° C.) from the abscess, from the blood, and from the peritoneum. Very free growth of the Staphylococcus aureus was obtained from

the first, but neither the cultivations nor cover-glass preparations from the blood or peritoneal cavity showed the presence of any bacteria whatever.

III. A guinea-pig inoculated with the same amount and material as the preceding, except that the inoculating needle was plunged deep into the abdominal cavity. Dead in ten hours. Examination showed only a marked redness about the point of inoculation, and that the needle had not penetrated the intestines. The heart was much distended with fluid blood. Cultivations were made from the point of inoculation, the peritoneal cavity, and the blood of the heart, and liver, and spleen, in gelatin and agar-agar. No growth appeared in any of the tubes except those sown from blood, and this growth proved to be the Staphylococcus aureus.

The contrast between the results of the last two experiments is very marked,—in the first case the organisms being found only in the abscess and not in the blood, and in the second case being only in the blood and not about the point of inoculation. In the first case the effect was purely local, and in the second it was general and of a septicæmic character.

IV. Staphylococcus pyogenes aureus from a perinephritic abscess (Case II.), seventeenth generation, cultivated for over a year. Two cubic centimetres of nutrient gelatin containing a culture were injected under the skin of the back of a guinea-pig. Killed in four days. An abscess

about an inch long by one-half inch broad, and three lines in thickness, full of thin yellow pus, was found just beneath the skin. Cultivations made from this showed the presence of the Staphylococcus aureus, and microscopic examination showed masses of micrococci in the pus and in the abscess-wall. Cultures from the blood gave no result whatever.

V. The same amount and material were employed as in the preceding experiment, and the inoculation was made subcutaneously over the abdomen. In two days death occurred. Section showed a very marked congestion of all of the abdominal viscera, with an abscess about one-half inch in diameter at the point of inoculation. Cover-glass preparations of the pus and sections of the abdominal wall showed the presence of masses of micrococci, whilst cultivations in nutrient gelatin and on agar-agar gave abundant growth of the Staphylococcus aureus. Cultures of the same organism were also obtained from the blood, and cover-glass preparations of the blood showed it to contain a number of micrococci.

VI. Staphylococcus aureus from a perinephritic abscess (Case IV.), eighteenth generation, cultivation extending over eight months. Two cubic centimetres of a culture in nutrient gelatin were injected into the abdominal cavity of a healthy guinea-pig. Killed on the fourth day. The animal was moribund. Section showed the presence of an extensive purulent perito-

nitis, and microscopic examination of the pus together with cultivations made from it demonstrated the presence of the Staphylococcus aureus. Cultures from the blood and microscopic examination of the same revealed absolutely nothing abnormal; no bacteria were found.

VII. Staphylococcus aureus. The same experiment as the preceding was performed in the same way, and with the same result, except that the animal was killed on the second day. The inflammatory process with the suppuration seemed to be, if anything, more extensive. The same results were obtained by microscopic and cultivation experiments; the Staphylococcus aureus was found in the pus but not in the blood.

VIII. Staphylococcus aureus from a psoas abscess (Case XIV.), the twelfth generation, cultivation out of the body extending over seven months. Four cubic centimetres of a solution in distilled water of one drop of a pure culture in nutrient gelatin were injected into the abdomen of a healthy guinea-pig. In two days a swelling the size of an English walnut could be felt under the point of inoculation. Killed at the end of twelve days. The remains of an abscess were found, the cavity having almost closed; only a few drops of thick yellowish pus were obtained. Microscopic examination of this, however, showed it to be a mass of micrococci, with a small proportion of cell-nuclei. Cultivations from this material gave only a feeble

growth, which was afterwards determined to be the Staphylococcus aureus. Cultures from the blood remained sterile.

IX. Staphylococcus aureus. The same generation and solution as in the preceding case were employed, but only one cubic centimetre was injected. There was no result observed. The animal was killed on the fourth day, when only a slight redness at the point of entrance of the inoculating-needle could be made out. Cultures made from the blood and from the peritoneum remained sterile.

X. Staphylococcus aureus. Two cubic centimetres of the solution used in the two preceding cases were injected into the knee-joint of a full-grown guinea-pig (right posterior). Death on the following day. Cultures from the blood and from the joint gave a free growth of the Staphylococcus aureus; the joint itself showed no suppurative action, but was the seat of intense redness, with great swelling and some infiltration of blood. A small vessel had undoubtedly been ruptured during the inoculation, and some of the infective material had in this way got into the blood-current.

XI. Staphylococcus pyogenes albus from an abscess at an ununited fracture of the humerus (Case I.), fifteenth generation, cultivated out of the body for thirteen months. Two cubic centimetres of a culture in nutrient gelatin were injected under the skin of the back of a full-grown guinea-pig. On the second day a slight swelling

was observed at the point of inoculation. The animal was killed on the fourth day, when section showed nothing abnormal in the abdominal or thoracic viscera. There was an abscess under the skin at the point of inoculation, about two centimetres in diameter, filled with quite fluid pus of a distinctly lighter color than that found in any of the preceding experiments. There was not much change in the tissues about the abscess; a very slight swelling, extending one or two millimetres from its boundaries, being all that was noticeable. Cultivations and microscopic examinations showed the presence of the Staphylococcus albus in the pus and in the abscess-wall, but not in the blood.

XII. Staphylococcus pyogenes albus. The same generation and cultivation were employed as in the preceding case. A drop of a culture in nutrient gelatin was placed in ten cubic centimetres of water, and two cubic centimetres of this were injected subcutaneously over the abdomen. The animal was killed on the fourth day, but no bacteria were found, and the point of entrance of the inoculating-needle was barely visible except upon close examination.

XIII. Staphylococcus pyogenes albus from a perinephritic abscess (Case II.), twelfth generation. In this and the following experiment, XIV., two cubic centimetres of a pure culture in nutrient gelatin were injected subcutaneously over the abdomen. Both guinea-pigs were killed

at the end of a week. In both were found small abscesses at the point of inoculation from one to three centimetres in diameter, and filled with a thin yellowish-white pus. Cultivations showed the presence of the Staphylococcus albus in both, but in neither of them was anything found in the blood.

XV. Staphylococcus pyogenes albus from a diphtheritic vagina (Case VI.), thirteenth generation, cultivated outside of the body for nine months. A minute portion of a cultivation in nutrient gelatin (one-half of a cubic centimetre), with two cubic centimetres of freshly-boiled distilled water, was injected subcutaneously in the right abdominal region of a guinea-pig. A small lump was felt under the point of inoculation upon the following day. The animal was killed at the end of ten days. The lump had apparently decreased in size, for upon section it was found to be barely as large as a pea, and contained a few drops of thin pus. Microscopic examination showed that there were large numbers of micrococci in the pus and in the walls of the abscess. Cultures in nutrient gelatin and upon agar-agar showed that they were the Staphylococcus albus.

XVI. This experiment was a simple repetition of the preceding. The same culture and the same amount were used in the same way, and the results were precisely the same.

XVII. Staphylococcus pyogenes albus, fourteenth generation, from a case of

diphtheria of the vagina (Case VII.), cultivated outside of the body for over nine months. One cubic centimetre of a solution of a culture upon agar-agar in distilled water was injected deep into the muscle of a leg of a guinea-pig. The animal was killed at the end of six days, when a clearly-defined abscess, about three centimetres in diameter, was found lying between the muscles, with practically no inflammatory tissue surrounding it. The pus was of the usual character, fluid, and of a rather light yellow color. Cultivations made from it gave colonies of the Staphylococcus albus, and microscopic examinations showed many micrococci in the pus and abscess-walls. Neither of these methods of investigation gave any result when applied to the blood and internal organs.

XVIII. Staphylococcus pyogenes albus. The same cultivation and from the same source was employed in this as in the preceding case. A larger amount, however,—five cubic centimetres,—was injected subcutaneously in the left abdominal region. The next day a very marked swelling could be seen, and upon the third day this swelling broke through the skin and commenced to discharge. Section showed a large abscess just below the point of inoculation. Cultivations gave the Staphylococcus albus and two varieties of bacteria, which were probably the result of contamination from the air, especially as sections of the abdominal wall showed micrococci only.

XIX. Staphylococcus pyogenes albus

cultivated from a psoas abscess (Case IX.), fifteenth generation, propagated outside of the body for more than nine months. One cubic centimetre of a culture in nutrient gelatin was injected under the skin of the back of a guinea-pig. The animal was killed on the sixth day, when an abscess the size of a small marble was found at the point of inoculation. Cultures from the pus gave a free growth of the Staphylococcus pyogenes albus; nothing was found in the blood and internal organs.

XX. and XXI. The same cultivation was used in the same quantity for inoculation into the abdomen of two other guinea-pigs. They were examined on the fifth day, and in both of them localized abscesses were found at the seat of inoculation, which were shown to contain the Staphylococcus pyogenes albus by cultivation experiments and by microscopic examination of the pus and of the abscess-walls.

XXII. Staphylococcus pyogenes albus from a psoas abscess (Case XV.), fourth generation, grown outside of the body for about three weeks. A guinea-pig was inoculated on the top of the head with two cubic centimetres of a culture of this organism in nutrient gelatin. A slight swelling the size of a pea made its appearance at the end of the first twenty-four hours. Examined on the third day, an abscess about two centimetres by three in dimensions was found just under the skin, and not involving any of the deeper tissues.

It contained the Staphylococcus pyogenes albus, as demonstrated by cultivation experiments.

XXIII. and XXIV. The same organism from the same source carried through four more generations, making the eighth, was injected into the abdominal cavity of two guinea-pigs, one cubic centimetre being used. Examination upon the seventh day. In one of the animals a small abscess, the size of a pea, containing a few drops of light yellow pus, was found lying just below the stomach. The abscess was clearly defined, and the neighboring structures were not involved. In the other animal an abscess about twice the size of the preceding was present just above and in front of the bladder. This also was as definitely defined as the other. Cultivation experiments showed the presence of the Staphylococcus pyogenes albus in both lesions.

XXV. Staphylococcus pyogenes albus (Case XVIII.) from an abscess of the inguinal glands, third generation, cultivated out of the body for three weeks. A guinea-pig was inoculated with one cubic centimetre of a culture of this organism in nutrient gelatin. The inoculation was subcutaneous and in the dorsal region. The animal was killed on the sixth day. An abscess three centimetres in diameter was found under the skin at the point of inoculation, and microscopic examination showed the walls and the pus to be full of micrococci, which upon cultivation were seen to be the Staphylococcus albus.

XXVI. The fourth generation of the same organism employed in the preceding experiment was used to inoculate a guinea-pig in the abdominal cavity, one cubic centimetre of the culture in nutrient gelatin being the quantity injected. It was killed on the third day, when, as in the other cases, an abscess was found lying in the abdominal cavity just anterior to the kidney and without implication of any of the surrounding tissues. Cultures from the pus it contained showed the presence of the Staphylococcus pyogenes albus; cultures from the blood gave no result.

XXVII. This experiment was the same as the preceding, and gave the same results in every particular.

XXVIII. Erysipelas coccus (Case V.), twenty-second generation, cultivated outside of the body for more than a year. A minute portion of a colony on nutrient agar-agar was diffused in sterilized distilled water, and two cubic centimetres of the mixture were injected into the skin of the top of the head of a guinea-pig, which had been previously shaved and washed with corrosive sublimate. A marked redness made its appearance on the following day, accompanied by much swelling of the skin. An incision into the skin was made, and cultivations from the edges of the wound gave a plentiful growth of the Erysipelas coccus.

XXIX. and XXX. The same generations and material were used in both cases. The inoculations were made subcutane-

ously in the abdominal region in guinea-pigs; they were followed by some swelling and tenderness of the skin and general languor and disinclination to movement. Section on the third day showed marked emaciation everywhere, and much congestion of the skin of the abdomen, with some œdema. Cultures made from the skin showed the Erysipelas coccus in abundance, and sections of the infiltrated tissue were found to contain numbers of these organisms lying in the lymph-channels and between the cells of the deeper layers.

XXXI. Staphylococcus pyogenes citreus (Case III.), twentieth generation, cultivated out of the body for over a year. Two cubic centimetres of a culture in nutrient gelatin were injected into the abdominal cavity of a guinea-pig. The animal was killed on the fourth day, when a slight infiltration about the point of inoculation was found, but no suppuration and no bacteria could be demonstrated anywhere in the animal's body.

XXXII. The same material in the same amount was employed for injection into the abdominal cavity of a second guinea-pig. The animal was killed on the second day, when an abscess the size of a pea was found among the intestines, which were adherent to its borders, and the whole abdominal cavity was much congested. Cultures from the pus in the abscess and from the blood gave colonies of the Staphylococcus pyogenes citreus.

XXXIII. A small portion of a culture of the same organism upon nutrient agar-agar was mixed with four centimetres of sterilized distilled water, and two centimetres of the mixture were injected under the skin of a guinea-pig in the abdominal region. The animal was killed on the third day, when an abscess about two centimetres in diameter was found at the seat of inoculation. Cultures from the pus of this gave colonies of the Staphylococcus citreus, but nothing was found in the blood.

XXXIV. Staphylococcus cereus flavus (Case XIV.), seventh generation, cultivated out of the body for six weeks. This organism is one described by Passet ("Untersuch. ü. die artifl. der eitrigen Phlegmonen des Menschen," Berlin, 1885), and which he considers to be active in the production of suppurative processes. He was, however, unable to produce any specific results in inoculation experiments with it, and the two which I made likewise gave negative results. The experiments were alike in character, and consisted in the injection into the abdominal cavity of guinea-pigs of two cubic centimetres of a culture in nutrient gelatin. Section of the first on the third day after the inoculation showed no result whatever, and cultivations from the blood and abdominal fluids failed to show the presence of any bacteria. The (XXXV.) second guinea-pig, killed on the second day, gave equally negative results, neither macro-

scopic, microscopic, nor bacteriological observations showing the presence of anything abnormal. The negative evidence to be offered in the case of this bacterium does not, therefore, permit us to say that it has any pathogenic properties which can be demonstrated by ordinary methods; the only fact which is known is that of its rather uncommon occurrence in suppurative processes. Passet's specimen was found in a case of chronic suppurative periostitis of the ulna in a phthisical male subject twenty-seven years of age. Mine occurred in the case of psoas abscess in a child which was supposed to be connected with disease of the spine.

XXXVI. Streptococcus pyogenes (Case XVI.), sixth generation, cultivated out of the body for six weeks. One cubic centimetre of a culture in nutrient gelatin was injected into the shoulder of a guinea-pig. In two days, examination showed extensive infiltration of the muscles about the point of inoculation, with free suppuration and a collection of pus in the axilla. Cultures from the places affected gave colonies of the Streptococcus, and microscopic examination of the pus showed the organism in large numbers.

XXXVII. and XXXVIII. One cubic centimetre of the seventh generation of the same organism was injected into the abdominal cavity of two guinea-pigs. They were found almost moribund on the morning after the inoculation. Section showed an extensive inflammation over

the whole abdominal cavity, with slight suppuration compared to the extensive nature of the process. Cultures from this region and from the blood of the heart gave abundant growth of the Streptococcus.

XXXIX. The same generation as was employed in the last two cases was used in this one. A very minute portion of a colony upon agar-agar was mixed with water and injected subcutaneously into a guinea-pig. Section upon the third day showed an extensive suppurative infiltration beneath the skin, extending out in all directions from the point of inoculation. The lymphatics all through the abdominal cavity were much congested, as were the spleen, liver, and kidneys. Cultures from the pus and from the blood in each of the organs, as well as from the heart, gave free growth of the Streptococcus pyogenes.

XL. Micrococcus pyogenes tenuis (Cases XVII. and XVIII.). Cultures of this organism were made as related in the account of the cases from which they were obtained. The fourth generation from (Case XVII.) an abscess of the arm was inoculated (one cubic centimetre of culture in nutrient gelatin), after cultivation for three weeks, subcutaneously in one guinea-pig, with no result. The third generation, from Case XVIII., a "bubon d'emblée," was injected in the same quantity and in the same way into a guinea-pig. Section in three days showed a very slight infiltration

of the tissues about the point of inoculation, but cultures from this situation remained sterile. Microscopic examination showed a few of the organisms lying in the infiltrated portion of the tissues; so very few, however, as to be exceedingly difficult of detection.

In speaking of Case XV.—a psoas abscess in a child—mention was made of an organism which was found in the cultivations from that source which presented certain peculiarities under cultivation which I believe have not yet been spoken of by other observers. It first attracted attention by the peculiar green fluorescence which it imparted to the gelatin culture-medium throughout the entire diameter of the test-tube, whilst it did not liquefy the gelatin, in this respect differing from the bacillus of green pus, which does produce liquefaction of that material. The only other organism with which it can be confounded by the macroscopic appearances of its cultures is the *fluorescent bacillus*, which produces the like pigmentation of the gelatin-culture medium. This color extends beyond the limits of the colony, and bacteria are not found in it, and it seems to be due to the action of some product of the growth of the organism upon the nutrient material. In fact, so far as I have yet investigated this bacterium there appears to be no difference between the appearances of its cultures in nutrient gelatin, agar-agar, and blood-serum, the main point being that, so far as I am aware, the fluo-

rescent bacillus has no pathogenic properties, whilst the organism which I am describing seems to have a very active influence upon guinea-pigs. Microscopically, the differences are marked, however, for this organism is a short bacillus, appearing singly, in pairs, and in chains from 0.3 μ to 0.4 μ in length, whilst very often its appearance in the tissues is that of a long rod, it being exceedingly difficult to decolorize the chain in this situation just enough to show the individual members. It grows well at the ordinary temperature of the room, much more rapidly at 37° C. It does not liquefy gelatin, but produces a grayish-white line in the track of a needle, and in a few days a marked greenish-yellow fluorescence extending throughout the substance of the nutrient material. On agar-agar it produces in from thirty-six to forty-eight hours a grayish-white colony, elevated above the surface of the material, which has a peculiar soft, glistening appearance. The same greenish-yellow fluorescence makes its appearance throughout the substance of the culture medium. Plate-cultures made in nutrient gelatin show round points of a whitish color in two days or more; the gelatin is not liquefied, and very soon presents the same greenish-yellow fluorescence as when the cultivations are made in test-tubes.

The limits of time at my disposal have been so narrow that I have not been able to investigate this organism as thoroughly as I should wish. What I have said of

it is merely a preliminary description, which I hope to fill out more in detail at a future time, and will merely mention, in addition, the inoculation experiments which I have made with it.

A guinea-pig was inoculated in the abdominal region (subcutaneously) with two cubic centimetres of the sixth generation of this organism in nutrient gelatin. It died on the following day. On section four to six hours after death, there was no localized suppuration, but the entire front of the body was as dry as if there had never been any fluids there. The skin could be readily lifted from the underlying tissues without any dissection whatever, and the muscles, particularly the pectorales, were as distinct from each other as if they had been carefully separated and all the intermuscular substance removed. In fact, the appearances were those of commencing putrefaction; there was a slight œdematous condition of the subcutaneous cellular tissue of the back. Cultures from this œdema, from the blood of the heart, and from incisions in various organs, gave colonies of the organism of which we are speaking. A little later, eight guinea-pigs were inoculated in the same way as the preceding, with the eighth generation of this bacterium. Section, in from four to six days, gave uniformly the result which is illustrated in these specimens. Nothing was found at the seat of the inoculation, but in every case the liver and spleen were

found to be enlarged and the seat of a greater or less number of small abscesses. Occasionally the kidney seemed to be somewhat swollen also, but no abscesses were to be seen. Cultivations from the blood and from the seat of inoculation always remained sterile. Colonies from these abscesses always produced the peculiar fluorescence and microscopic appearances which I have attempted to describe. These include all the experiments which I have yet made in this direction. I have thought it well to speak of them here, because they seem to bear out my assertion that the end is not yet in regard to the isolation of new species of bacteria connected with the suppurative processes in man, and because I thought it might interest the members of this Association to have these facts presented to them.

The conclusions which may be drawn from the work here reported are as follows:

I. The cultivation experiments conducted over so long a space of time, with the successful inoculations at the end of that time, indicate very plainly the retention of the pathogenic power of these organisms indefinitely. Their virulence is just as great at the end of a year, and after being passed through a large number of generations, as it is when taken from their original birthplace.

II. Their permanence of form is also well demonstrated, their microscopic appearances and peculiarities under cul-

tivation undergoing absolutely no alteration.

III. In order to obtain either a modification of their pathogenic powers or of their morphological properties,—if this is possible at all,—some different methods of observation must be used than those which have hitherto been employed.

IV. The probabilities indicate that work in this direction is not likely to be attended with much success: *a*, because no modifications have yet been observed, and, *b*, because prophylaxis furnishes the easiest and most generally applicable safeguard against the activity of these lower forms of life.

V. So far as the experiments go, they tend to show that no form of the suppurative process in man is unattended by bacteria, and that the inoculation in the lower animals of pure cultures of these bacteria is followed by more or less acute and extensive suppuration.

VI. This conclusion—that of the dependence of the suppurative process upon the activity of bacteria—is very strongly supported by the most recent evidence of the best equipped workers in the field of bacteriological research.

VII. Very different clinical phenomena may be produced by the same organism; all of these phenomena, however, coming under the general head of the inflammatory and suppurative processes.

VIII. The differences in the results produced by the same micro-organism in

different individuals depend upon causes foreign to the bacterium itself.

IX. These differences are the result of differences in the amount of the infectious material received into the system, and of the locality or lesion by which it gains access, and also by variations in individual condition,—the "personal equation" being a very large factor in making up the sum of any results in bacteriological work.

Certain investigations upon the resistance to various destructive reagents of some of these forms of bacteria have been made by Passet (*l. c.*). The organisms used were mainly the Staphylococcus pyogenes aureus and the Streptococcus pyogenes, these being selected as the most common and the most active of their class; and the reagents employed were salicylic and carbolic acids and corrosive sublimate. The results indicated the superiority of corrosive sublimate as a destructive agent, six drops of a one-per-cent. solution of this material preventing the development of either of the bacteria mentioned in ten cubic centimetres of nutrient gelatin, whilst it required twenty-five drops of a five-per-cent. solution of carbolic acid or one hundred drops of a solution of salicylic acid (one to three hundred) to do the same thing.